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Research Note—

Eye Surface Area and Dosage Rates for Spray Vaccination

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SUMMARY. Spray application of *Mycoplasma gallisepticum* (MG) vaccines is a labor- and time-saving means of mass vaccination of layer chickens. Recent assessment of spray characteristics of nozzles commonly used to apply MG vaccine in layer chicken operations has shown that the amount of respirable droplets (<5 μm) is negligible. Topical application of vaccine onto the eye surface has been suggested as a route of vaccination, but no estimates of vaccine load delivered via spray application were found in the literature. Estimates of eye surface area were developed using digital imaging; 24 layer pullets were used for analysis, and the mean eye surface area, corrected for corneal curvature, was found to be 0.609 cm^2. This surface area was then used to estimate vaccine load for commercially available live MG vaccine sprayed through popular nozzles. Less than 3000 colony-forming units can be expected for direct deposition onto the surface of an eye.

RESUMEN. *Nota de Investigación*—Área de la superficie ocular y dosis para la vacunación en aerosol.

Las aplicación por aerosol de la vacuna contra *Mycoplasma gallisepticum* es una forma de ahorrar mano de obra y tiempo en la vacunación masiva de las aves de postura. La evaluación reciente de las características de las boquillas para aerosoles comúnmente utilizadas para aplicar la vacuna contra *M. gallisepticum* en las operaciones de aves de postura ha demostrado que la cantidad de gotas respirables (menores de 5 micras) es insignificante. Se ha sugerido la aplicación tópica de la vacuna en la superficie del ojo como una vía de vacunación, pero no hay estimaciones en la literatura acerca de la carga vacunal que se distribuye a través de la aplicación por aspersión. Se desarrollaron estimaciones del área de la superficie ocular utilizando la digitalización de las imágenes; se utilizaron 24 pollitas de postura para este análisis. La superficie ocular media corregida de acuerdo a la curvatura de la córnea, se determinó es de 0.609 cm^2. Esta superficie fue utilizada para estimar la carga de vacunas para las vacunas vivas de *M. gallisepticum* disponibles comercialmente, que se aplican por aerosol utilizando las boquillas habituales. Se puede esperar menos de 3000 unidades formadoras de colonias que sean depositadas directamente sobre la superficie de un ojo.

Key words: spray vaccination, vaccine optimization, dosage rate, layer chicken, mycoplasmosis

Abbreviations: CESA = corrected eye surface area; cfu = colony-forming units; CROC = corneal radius of curvature; CSA = corneal surface area; HG = Harderian gland; MG = *Mycoplasma gallisepticum*; NCA = noncorneal area; PCA = projected cornea area; PCD = projected cornea diameter; PEA = projected eye area; φ = included angle of corneal diameter (°)

Spray application of commercially available live *Mycoplasma gallisepticum* (MG) vaccines is a labor- and time-saving means of mass vaccination of layer chickens (7). The effectiveness of spray application of live MG vaccines to poultry can be affected by numerous factors including vaccine suspension titer (13), chemical properties of the suspension media (22,23), temperature of the suspension media (6), physical characteristics of the spray (29), and temporal effects on viability after resuspension (6,22).

Routes of vaccination via spray application may include inhalation through the nares with subsequent travel into the respiratory system, topical application onto the eye surface and associated adnexa, and ingestion (9). While inhalation through the nares, with subsequent transport through the remainder of the respiratory system, is typically cited as a means of vaccine uptake, the particle size necessary to traverse the respiratory tract to its lower recesses is less than 5 μm (11,16). Hayter and Besch (16) showed that particles which averaged 5 μm or larger were deposited primarily in the upper respiratory system; hence, droplets of vaccine suspension must be smaller in order to be transported into the lower respiratory system. More recently, Corbanie *et al.* (11) evaluated the transport of particles ranging in size from 1 to 20 μm through the respiratory system in broilers aged 1 day, 2 wk, and 4 wk. The majority of particles 5 μm or greater were found associated with the eyes and nares at least 70% of the time.

Topical application of vaccine onto the surface of the eyes results in drainage into the nasal passages via the nasolacrimal duct (25). Within the nasal passages, most of the lymphoid tissue is present around the choanal and infundibular clefts just rostral to the pharyngeal papillae (15,20,21,32). Vaccine uptake may occur via the secretory duct of the Harderian gland (HG), which connects it to the nictitating membrane (8). The main source of IgA in tears is derived from the HG, and the HG may influence the humoral immune response in other mucosal sites because HG-derived IgA+ B cells have been shown to migrate selectively to cecal tonsils (34). Finally, ingestion of vaccine results in potential stimulation in the upper gastrointestinal tract of lymphoid tissue in the cervical and thoracic parts of the esophagus as well as in the esophageal tonsil and lymphoid tissue in the proventriculus (10).

Because a recent assessment of spray characteristics of nozzles commonly used to apply live MG vaccine in layer chicken operations has shown that the amount of respirable droplets (<5 μm) is negligible (29), it is reasonable to assume that the preponderance of both mucosal and systemic immune responses

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attributable to vaccination with the particular spray apparatus described herein results from stimulation of secondary lymphoid organs, including the HG-derived B cells and the nasal-associated lymphoid tissue in the upper respiratory tract, particularly in the nasal cavity and the nasolacrimal duct (27).

The nozzles commonly used to apply live MG vaccine in commercial layer chicken operations generate a negligible amount of respirable droplets (<5 μm) (29); all three nozzles tested were classified as "Very Fine" spectra according to published standards (4). Only 10% of droplets generated were smaller than 94.6 μm, with a maximum of 0.35% of the spray having a droplet size of less than 10 μm. Hence, it is reasonable to assume that, for spray application of live MG vaccine in current commercial practice, the most likely route of vaccination is via the eye, either through direct deposition incurred during the vaccination process or via subsequent secondary contact.

In order to estimate the amount of vaccine that a bird may receive via spray on the surface of an eye, the deposition (volume deposited per area), surface area of the eye, and vaccine solution titer must be known. Purswell *et al.* (29) measured deposition of commonly used spray nozzles for three different nozzle types and two different system pressures and found the average deposition ranged between 0.01 and 1.07 μl/cm^2; thus, deposition is likely the most important factor to consider when spray-applying vaccines of any sort. The best performing nozzle produced a droplet size spectrum with a median volume diameter of 161 μm, covering 26.2% of exposed area, and a resultant deposition of 0.99 μl/cm^2 for a spray path of 76 cm, which is the nominal distance from the nozzle to birds in commercial caged housing systems. No estimates of eye surface area for chickens or other birds were found in the literature; thus, the objective of this study was to develop estimates of projected eye surface area and dosage rates from commonly used nozzles for 10-wk-old layer pullets.

MATERIALS AND METHODS

Twenty-four Hy-Line W-36 pullets were obtained from a commercial hatchery. Pullets were group-housed in floor pens until 9 wk of age. Pullets were moved into battery cages in an environmentally controlled room where conditions were maintained at 21 C and 50% relative humidity. The lighting schedule followed the one recommended by the primary breeder (17). All procedures were approved by the Mississippi State location USDA-ARS Animal Care and Use Committee.

Photogrammetric analysis was used to obtain dimensional information about the left eyes of layer pullets. Photogrammetry is defined as the generation of geometric or dimensional data from images and has traditionally been used in remote sensing applications such as surveying and geomatics (3,28), but has also been adapted to biomedical applications (24) and inspection during manufacturing (5), as well as agricultural applications such as estimating body dimensions of pigs (35) and dimensions of grain particles (18).

Estimation of eye surface area was carried out using the process shown in Fig. 1. Pullets were photographed at 10 wk of age, corresponding to the typical age at which commercial pullets are spray vaccinated with live MG vaccine. Pullets were placed into a positioning cradle (Fig. 1) with a dark background to provide contrast. A digital camera with a 6-megapixel resolution was mounted on a tripod 60 cm from the head of the bird. Positions of the camera and birds were held constant to negate any effects from changes in relative positioning. Two circular reference targets (diameters of 12.84 and 19.14 mm) were imaged to determine the linear and area scale factors used to transform all measurements made on the images into physical dimensions. The linear scale factor and area scale factor for the images used in this analysis were 0.0390 mm/pixel and 0.0015 mm^2/pixel^2, respectively. The images were acquired with the camera and then transferred to a computer (Optiplex 755, Dell, Inc., Round Rock, TX) for analysis.

Images were analyzed using ImageJ, a public domain image analysis application available from the U.S. National Institutes of Health (1,30). The polygonal selection tool was used to trace the periphery of the eyelid (Fig. 1); this operation was repeated three times for each image of each bird. Projected eye area (PEA) was then determined using the "Measure" command which enumerates the number of pixels contained in the outlined area. Pixel areas were translated into physical dimensions by multiplying by the area scale factor (0.0015 mm^2/pixel^2).

Estimation of corneal surface area. Total exposed eye surface includes both the area covered by the cornea, which protrudes from the eye, as well as the sclera. Measurement of projected area treats the surface as flat and, thus, must be corrected for protrusion of the cornea. In order to correct for the spherical area of the cornea, the projected area of the cornea must be subtracted from the PEA. Projected cornea diameter (PCD) was determined by using the straight-line selection tool and the "Measure" command; diameter lengths were scaled using the linear scale factor (0.0390 mm/pixel). Projected cornea area (PCA) was then calculated using the equation for area of a circle:

$$\mathrm{PCA} = \frac{\pi \times \mathrm{PCD}^2}{4}. \tag{1}$$

Noncorneal area (NCA) was then calculated using Equation 2:

$$\mathrm{NCA} = \mathrm{PEA} - \mathrm{PCA}. \tag{2}$$

The area of the spherical section representing the cornea can be determined using geometric relationships combined with the NCA to correct eye surface area estimates for the curvature of the cornea.

The chicken eye is aspherical and, as such, corneal radius of curvature (CROC) increases approximately 3%–4% from the pupillary axis towards the pupillary margin (31). CROC also changes with age (18,31) and is larger in male chickens as compared to females (31). However, only single-point measurements of CROC have typically been reported in the literature (14,19,33). Estimates for CROC were taken from Schaeffel and Howland (31), which provided the following relationship for estimating CROC as a function of age in female chickens:

$$\mathrm{CROC} = 0.025 \times \mathrm{Age} + 2.94, \tag{3}$$

where Age = bird age in days.

While CROC does vary across the surface of the eye, the variation is minimal (3%–4%) and, thus, the corneal surface area (CSA) of the chicken eye was assumed to be spherical for the purposes of estimating surface area. The surface area of a spherical section can be calculated by Equation 4 from Oberg *et al.* (26), using CROC as the radius of a sphere; the general geometric arrangement of the avian eye is shown in Fig. 2.

$$\mathrm{CSA} = 2 \times \pi \times \mathrm{CROC}^2 \left[1 - \cos\left(\frac{\varphi}{2}\right)\right], \tag{4}$$

where CSA = corneal surface area (cm^2); CROC = corneal radius of curvature (cm); and φ = included angle of corneal diameter (°).

The included angle of the corneal diameter (φ) is unknown, but can be calculated using the projected chord length of the cornea (taken as PCD herein) and CROC, with the relationship for calculation of chord length for a circular section (26):

$$\mathrm{PCD} = D \sin\left(\frac{\varphi}{2}\right) = 2 \times \mathrm{CROC} \times \sin\left(\frac{\varphi}{2}\right), \tag{5}$$

where: PCD = projected cornea diameter (cm); D = diameter of circle (cm); CROC = corneal radius of curvature (cm); and φ = included angle of corneal diameter (°).

Rearranging Equation 5 yields:

$$\varphi = 2 \times \sin^{-1}\left(\frac{\mathrm{PCD}}{2 \times \mathrm{CROC}}\right). \tag{6}$$

Substituting Equation 6 into Equation 4, CSA can be estimated using published estimates of CROC and photogrammetric measurements of corneal section chord length:

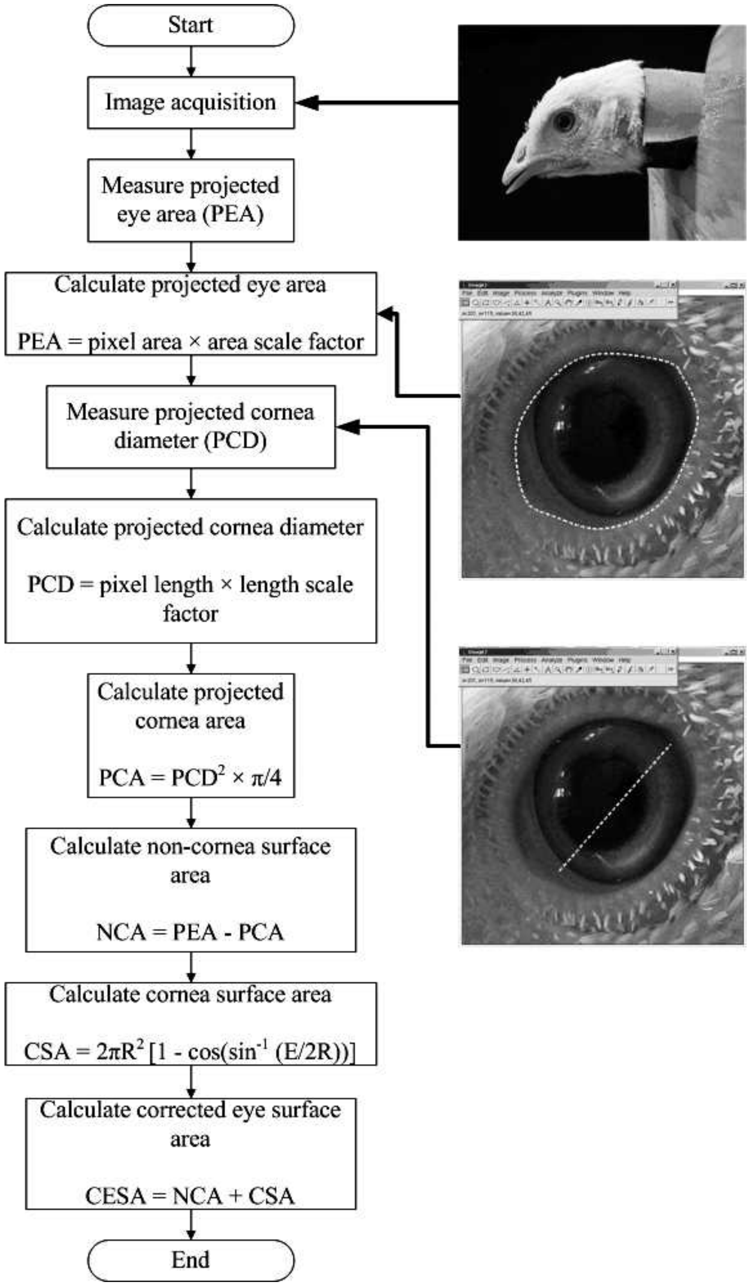

Fig. 1. Analysis procedure for estimation of corrected eye surface area.

$$CSA = 2 \times \pi \times CROC^2 \left[1 - \cos\left(\sin^{-1}\frac{PCD}{2 \times CROC}\right)\right]. \tag{7}$$

The sum of CSA, estimated by Equation 7 and NCA, is reported as estimates of corrected eye surface area (CESA).

Dosages from popular spray nozzles were calculated using deposition measurements reported by Purswell *et al.* (29). Deposition and flow rate from three popular commercially available spray nozzles for two system pressures, as reported by Purswell *et al.* (29), are shown in Table 1. Vaccine load received is determined by the product of vaccine titer, eye surface area, and deposition. The titer of commercially produced live MG vaccine (F-VAX MG®, Schering-Plough Animal Health, Omaha, NE) is typically in the range of 2×10^6 colony-forming units (cfu)/dose (12).

Orientation of the bird relative to the path of the spray nozzle will also affect the amount of vaccine topically applied on the eye surface. The incident angle describes the angle from which droplets approach the surface of the eye. Maximum deposition onto the eye surface will occur when the spray plume is perpendicular to the eye surface (90°); little, if any, deposition should be expected when the surface of the eye is parallel to the spray plume. Incident angle of the spray plume will also affect the proportion of the spray reaching the eye. Adjustments for angle were calculated by the proportional reduction in projected area, for a range in angles of 0° (parallel) to 90° (perpendicular), using the product of the sine of the incident angle and eye surface area.

RESULTS

Results for photogrammetric measurements (projected surface area and PCD), and subsequent calculations of geometric parameters

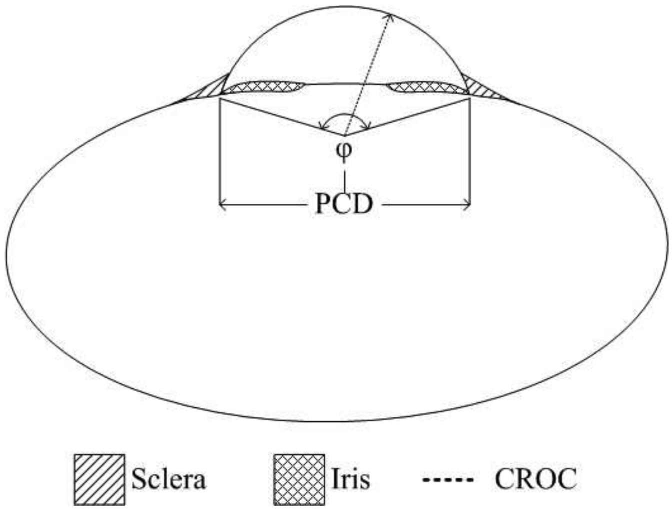

Fig. 2. Conceptual geometry of the avian eye. CROC = corneal radius of curvature; PDC = projected corneal diameter; and φ = of corneal diameter (°).

for each of the 24 pullets, are shown in Table 2. The mean CESA for all 24 pullets was 0.609 cm^2 with a 95% confidence interval of 0.051 cm^2. The data show that PEA, which was measured directly from the images, yielded lower estimates of eye surface area for all 24 pullets; the mean difference between the PEA and CESA was 17.8%.

Vaccine load delivered to an eye of the mean corrected eye surface area (0.609 cm^2) was estimated, using the deposition data reported by Purswell *et al.* (29) for high (448.1 kPa) and moderate (310.2 kPa) system pressures, for three types of nozzle commonly used for poultry vaccine application. Estimated vaccine load ranged from 26 to 2809 cfu/eye for the mean CESA (Table 3). For comparison, the spray application volume for an eye is approximately 1/711th of the full dose of 2×10^6 cfu.

Table 1. Flow rate and deposition for commercially available nozzles used for spray application of vaccine. Data adapted from Purswell *et al.* (29).

Nozzle	Pressure (kPa)	Flow rate (ml/min)	Deposition (μl/cm^2)
Coarse (1553-10)	310.2	536.8	0.91
	448.1	684.4	1.07
Medium (1553-08)	310.2	209.2	0.24
	448.1	249.5	0.07
Fine (1531-06)	310.2	244.0	0.01
	448.1	288.6	0.02

Orientation of the bird relative to the spray path will affect the amount of exposed area available for deposition of vaccine onto the eye surface. Proportional reductions in exposed area and nominal vaccine load were calculated for a range of incident angles for a coarse nozzle, at both high and moderate system pressures, and the trends are shown in Fig. 3. Incident angles greater than 73.5° provide deposition of greater than 90% of maximum application volume when the spray nozzle is perpendicular with the eye surface, and the incident angle must be reduced below 25° to provide less than 50% of maximum application volume.

DISCUSSION

Spray application of vaccine is a common practice in the commercial poultry industry for convenience, labor savings, and

Table 2. Geometric properties of pullet eyes as measured via photogrammetry. Table contents reflect the mean of three measurements for each bird. (PEA = projected eye area, PCD = projected cornea diameter, PCA = projected cornea area, NCA = non-corneal area, CSA = cornea surface area, and CESA = corrected eye surface area).

Bird	PEA (cm^2)	PCD (cm)	PCA (cm^2)	NCA (cm^2)	CSA (cm^2)	CESA (cm^2)
1	0.433	0.712	0.398	0.034	0.483	0.517
2	0.460	0.702	0.387	0.073	0.466	0.538
3	0.448	0.736	0.425	0.023	0.524	0.548
4	0.422	0.695	0.379	0.042	0.454	0.496
5	0.439	0.699	0.384	0.055	0.461	0.516
6	0.350	0.650	0.332	0.018	0.386	0.404
7	0.494	0.707	0.393	0.101	0.474	0.575
8	0.474	0.676	0.359	0.115	0.424	0.540
9	0.469	0.705	0.391	0.078	0.471	0.549
10	0.495	0.717	0.404	0.092	0.491	0.583
11	0.456	0.752	0.444	0.013	0.555	0.568
12	0.500	0.750	0.442	0.057	0.553	0.610
13	0.530	0.736	0.425	0.105	0.525	0.630
14	0.523	0.737	0.426	0.097	0.526	0.623
15	0.514	0.774	0.471	0.044	0.601	0.645
16	0.503	0.750	0.442	0.061	0.552	0.613
17	0.478	0.707	0.392	0.085	0.473	0.559
18	0.565	0.823	0.532	0.033	0.718	0.752
19	0.495	0.719	0.407	0.088	0.495	0.583
20	0.645	0.904	0.642	0.004	1.013	1.017
21	0.625	0.836	0.549	0.077	0.755	0.831
22	0.608	0.833	0.544	0.064	0.745	0.809
23	0.492	0.717	0.403	0.089	0.490	0.579
24	0.451	0.718	0.405	0.045	0.493	0.539
Mean	0.495	0.740	0.432	0.062	0.547	0.609
SD	0.066	0.058	0.070	0.031	0.137	0.129

Table 3. Estimated applied volume and dosage for overall mean corrected eye surface area (0.609 cm^2) for a single eye of a 10-wk-old commercial layer chicken for typical commercial MG vaccine titer. Model numbers for each nozzle type listed with the type description; all nozzles are available from HARDI (Davenport, IA).

	Applied volume (µl)		Vaccine load (cfu)	
Nozzle	310.2 kPa	448.1 kPa	310.2 kPa	448.1 kPa
Coarse (1553-10)	0.554	0.652	2389	2809
Medium (1553-08)	0.146	0.043	630	184
Fine (1531-06)	0.006	0.012	26	52

effectiveness. However, the dynamics of vaccine uptake via spray application are not well understood. Determining the partitioning of the routes of vaccine uptake is necessary for an accurate estimate of the realized dosage of vaccine received; this is needed to optimize the amount of vaccine applied to a flock of commercial layer pullets in order to provide adequate protection from disease challenge in a cost effective manner. Possible routes of spray-applied vaccine uptake include topical application of vaccine solution on the surface of the eye, inhalation, and ingestion. Inhalation is limited in effectiveness with current spray application technology due to large droplet sizes. Thus, it is likely that deposition onto body surfaces is a key means of delivery. The eye has been suggested as a route of vaccine uptake, given its connection to the respiratory system (8,20). Physical contact with other birds has been cited as a route of vaccine uptake for spray application of Newcastle disease vaccine (2).

The exposed eye surface comprises a very small proportion of the total exposed surface area of the body of a chicken; thus, the dosage received directly through this route would be significantly less when compared to the amount of vaccine solution applied to the remaining body, indicating that indirect exposure (i.e., contact with other birds) may be an important component in vaccine application. Alexander (2) suggests that indirect exposure from other birds is indeed a means of exposure in spray vaccination for Newcastle disease. In addition, orientation of the bird relative to the spray path will dictate the amount of exposed area available for deposition of vaccine onto the eye surface. As the incident angle approaches 0° (parallel), vaccine deposition onto the eye surface is minimized, and no vaccine deposition should be expected onto the eye surface when the head of the bird is not facing the spray plume. Deposition of vaccine spray on the surface of the eye increases until the spray plume is perpendicular to the eye surface, and this relationship is nonlinear. Droplet sizes from popular spray nozzles used for application of live MG vaccine showed large differences in volumetric flow rate and deposition (29). Increased deposition improves coverage and, thus, volumetric flow rate would be an important consideration in maximizing vaccine application via indirect exposure.

The immune response of chickens to MG is known to be dose-dependent (13). The serum plate agglutination test exhibits a more-rapid positive response in commercial layers that are eye-drop vaccinated as compared to commercial layers which are spray vaccinated with live MG vaccine (data not shown). The results of the present study suggest that the 1/711th of the full (eye-drop) dose of 2×10^6 cfu may not include the totality of organismal exposure in spray-applied live MG vaccinated hens. Future research should

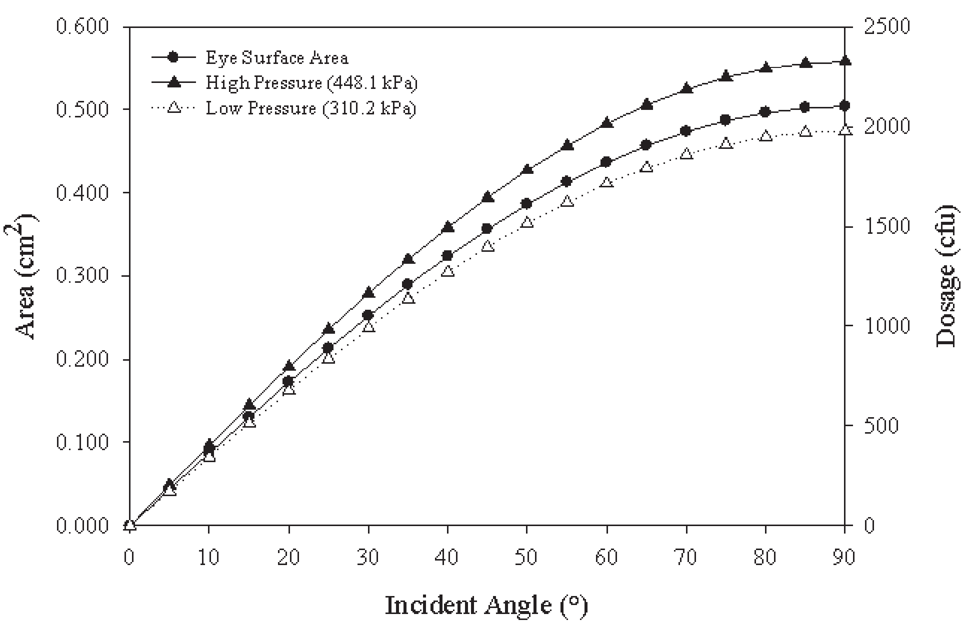

Fig. 3. Variation in estimated exposed eye surface area and vaccine load for a coarse nozzle, as influenced by the angle of the spray plume to the eye.

provide insight and understanding as to the contribution of different modes of vaccine uptake (ingestion, inhalation, or HG stimulation) on the subsequent seroconversion of layer chickens to spray-applied live MG vaccine.

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